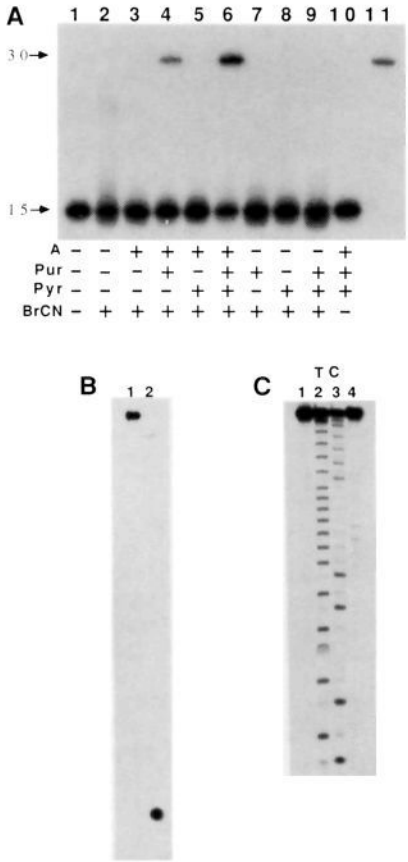

Figure 3. Condensation of oligonucleotides A and (^{32}P)B and product analysis; autoradiograms of high-resolution, 20% polyacrylamide denaturing gels: (A) Production of a radioactive 30-mer by template-directed condensation of 15-mers A and (^{32}P)B. Lanes 1–10: all reaction mixtures contain 5′-^{32}P-phosphorylated B. Activation of the 5′-phosphate of B was initiated by addition of a freshly prepared solution of BrCN (0.4 M) to the reaction mixtures. Reaction mixtures initially contained 40 mM BrCN, 20 mM imidazole·HCl (pH 7.0), 20 mM $NiCl_2$, and 100 mM NaCl in a total volume of 30 μL. In control experiments, BrCN, oligonucleotide A, and one or both strands of the template were omitted from the reaction. After a 9-h reaction time (20 °C), the DNA was precipitated with ethanol, and the precipitates were dissolved in buffer and loaded on the gel. Lane 11: authentic synthetic 30-mer with the sequence 5′-$T_4(CT)_2T_2(CT)_2T_6(CT)_5$-3′ phosphorylated at its 5′ end with polynucleotide kinase and [γ-^{32}P]ATP. (B) Enzymatic hydrolysis of the condensation product by treatment with calf spleen phosphodiesterase. Lane 1: condensation product. Lane 2: condensation product after treatment with 4 μg of calf spleen phosphodiesterase (Boehringer Mannheim) at 37 °C for 8 h. (C) Sequencing analysis of the 5′-phosphorylated condensation product. Lane 1: purified, ^{32}P 5′ end-labeled condensation product. Lane 2: T-selective chemical cleavage reaction ($KMnO_4$).[15] Lane 3: C-selective cleavage reaction (hydrazine, NaCl).[16] Lane 4: 5′ end-labeled condensation product treated with 4 μg of calf spleen phosphodiesterase at 37 °C for 8 h.

containing most of the radioactive label and the absence of a radioactive 16-mer corresponding to enzymatic termination at the coupling site indicate that the predominant linkage formed in the condensation reaction is a 3′,5′-phosphodiester bond.

For sequencing analysis, the condensation reaction was carried out with oligodeoxynucleotide B phosphorylated by using unlabeled ATP. The A–B product was then purified by gel electrophoresis and treated with polynucleotide kinase and [γ-^{32}P]ATP. The ^{32}P 5′ end-labeled product was isolated by gel electrophoresis and subjected to chemical sequencing reactions specific for C and T (Figure 3C, lanes 2 and 3).[15,16] The sequence of the 30-mer verifies the condensation of the 3′ terminus of A with the 5′ terminus of B. Finally, the 5′-phosphorylated product was treated with calf spleen phosphodiesterase and shown to be resistant to degradation, confirming that the exonuclease had little endonuclease or 3′-exonuclease activity (Figure 3C, lane 4).

In conclusion, double-stranded DNA can serve as a template to align reactive termini of oligonucleotides and promote their condensation. Within the context of self-assembling chemical systems for macromolecular information transfer, this conversion of sequence information is not self-replicating.[17] Triple-helix-directed ligation can create sequences that are neither identical nor complementary in a Watson–Crick sense to the template, but rather *new sequences* of nucleic acids.

Acknowledgment. This work was supported in part by the National Institutes of Health (GM-35724) and the Caltech Consortium in Chemistry and Chemical Engineering (founding members: E. I. du Pont de Nemours and Company, Inc., Eastman Kodak Company, Minnesota Mining and Manufacturing Company, and Shell Oil Company Foundation). We are grateful for a National Research Service Award to K.J.L. from the National Institute of General Medical Science.

(15) Maxam, A. M.; Gilbert, W. *Methods Enzymology* **1980**, *65*, 497–559.
(16) Kochetkov, N. K.; Budowskii, E. I. *Organic Chemistry of Nucleic Acids*; Plenum: New York, 1972; Part B, pp 412–416.
(17) (a) Orgel, L. E. In *Cold Spring Harbor Symp. Quant. Biol. 1987*; Cold Spring Harbor; Vol. L11, p 9. (b) Joyce, G. F. In *Cold Spring Harbor Symp. Quant. Biol. 1987*; Cold Spring Harbor; Vol. L11, p 41.

Chelation Enhanced Fluorescence Detection of Non-Metal Ions

Michael E. Huston,[1] Engin U. Akkaya, and Anthony W. Czarnik*

Department of Chemistry
The Ohio State University
Columbus, Ohio 43210
Received May 1, 1989

Several groups have reported observing fluorescence enhancements in nonaqueous solutions upon the binding of some metal ions to conjugate fluorescent probes, probes in which the metal ligand is not an integral part of the fluorophore substructure.[2] Conjugate probes offer great potential as reporter molecules in that the known selectivities of azacrown and cryptand binding sites can, in principle, be applied unaltered to the design of fluorescent probes with similar selectivities and large signal ranges. Recently, we have found that anthrylazamacrocycles demonstrate both chelation-enhanced fluorescence (CHEF) and chelation-enhanced quenching (CHEQ) upon metal ion binding in 100% aqueous solution.[3] However, both conjugate and integral fluorescent probes have been applied to date almost exclusively to the detection of metal ions.[4] We now report the first observation of large CHEF effects on the binding of anions to anthrylpolyamine conjugate probes, which serve to "signal" molecular recognition events involving carboxylate, sulfate, and phosphate groups. Moreover, we propose intracomplex protonation of a

(1) Recipient of an Ohio State University graduate fellowship and an Amoco Fellowship.
(2) (a) Sousa, L. R.; Larson, J. M. *J. Am. Chem. Soc.* **1977**, *99*, 307. (b) Konopelski, J. P.; Kotzyba-Hibert, F.; Lehn, J.-M.; Desvergne, J.-P.; Fages, F.; Castellan, A.; Bouas-laurent, H. *J. Chem. Soc, Chem. Commun.* **1985**, 433. (c) Street, K. W., Jr.; Krause, S. A. *Anal. Lett.* **1986**, *19*, 735. (d) de Silva, A. P.; de Silva, S. A. *J. Chem. Soc, Chem. Commun.* **1986**, 1709. (e) Huston, M. E.; Haider, K. W.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 4460. (f) Ganion, S. J.; Stevenson, R. W.; Son, B.; Nikolakaki, C.; Bock, P. L.; Sousa, L. R. *Abstracts of Papers*, 197th National Meeting of the American Chemical Society, Dallas, Texas, April 1989; American Chemical Society: Washington, DC, 1989; 0133, Organic Division.
(3) Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. Submitted to *J. Am. Chem. Soc.*
(4) The single exception is found in a stimulating paper by Lehn (*J. Chem. Soc., Chem. Commun.* **1988**, 596) in which the binding of triphosphate was detected with a receptor molecule that itself binds ATP and catalyzes its hydrolysis; upon the binding of triphosphate at pH 7.6, a 14% decrease in fluorescence was observed.

0002-7863/89/1511-8735$01.50/0

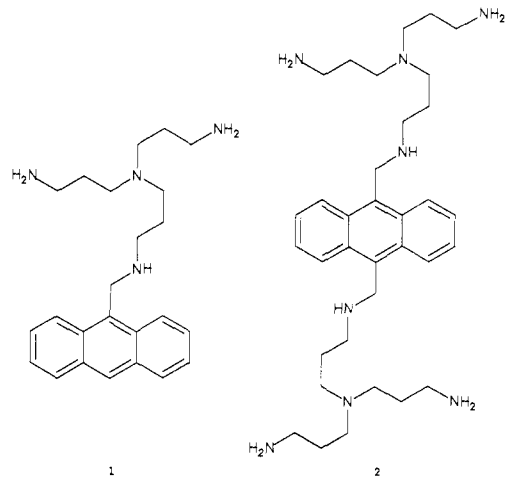

Figure 1.

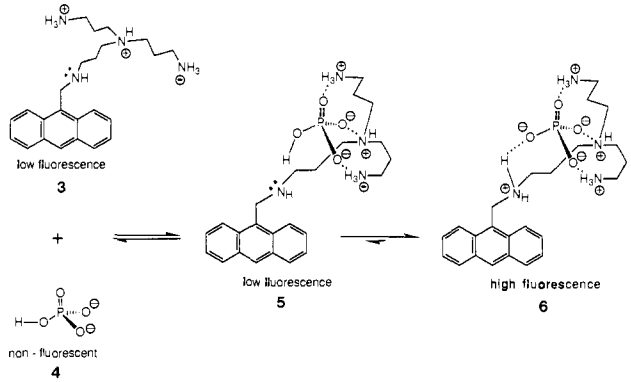

Figure 2.

quenching nitrogen, facilitated by the bound guest, as the structural basis for these CHEF effects.

Anthrylpolyamines **1** and **2** (Figure 1) were synthesized by the reaction of tris(3-aminopropyl)amine[5] with 9-(chloromethyl)-anthracene[6] and 9,10-bis(chloromethyl)anthracene,[6] respectively, using an adaptation of a literature procedure.[7] Both compounds were isolated and characterized as their HCl salts.[8]

Examination of the literature leads to the conclusion that a change in protonation or chelation state of a benzylic nitrogen leads to large fluorescence enhancements.[9] Thus, trication **3** (Figure 2) is the ionic form of **1** that can act as an anion probe at pH 6.[10] As shown in Figure 2, complexation of the anionic

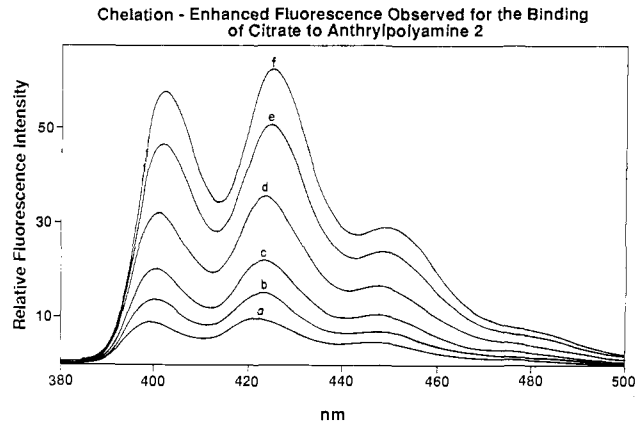

Figure 3. Fluorescence spectra for 5 μM solutions of **2** in the presence of increasing concentrations of citrate: (a) none, (b) 0.1 mM, (c) 1 mM, (d) 10 mM, (e) 0.1 M, (f) 0.2 M. Excitation was performed at 335 ± 3 nm. All solutions were adjusted to pH 6.

phosphate oxygens[11] with ammonium ions on **3** places the remaining phosphate OH group in close proximity to the free amine; this species (**5**) will demonstrate low fluorescence due to quenching by the free amine group. However, favorable intracomplex proton transfer will lead to **6** in which intramolecular quenching is eliminated and higher fluorescence is observed. Evidence in support of this hypothesis is found in the pH profile of the CHEF effect, which shows a bell-shaped response curve closely matching the calculated curve for appearance of the trication of **3** at various pH's. Our analysis using the HPO_4^{2-} ion as shown in Figure 2 illustrates the general principle by which CHEF is seen for anions. Species such as sulfate and acetate, which yield smaller fluorescence enhancements upon binding, are totally dissociated at pH 6 and thus cannot deliver a proton directly; however, both anions are capable of stabilizing a proximal ammonium ion via bridging as in structure **6**.[12]

Fluorescence spectra were recorded for aqueous solutions[13] of **1** in the presence of increasing concentrations of several anions. With use of the above model the concentration of complex **6** was calculated for each solution of known total anion concentration.[14] The effective binding constant of phosphate[15,16] to **3** (log K_{eq} =

(5) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186.

(6) Purchased from Aldrich Chemical Company, Inc., 940 West Saint Paul Ave., Milwaukee, Wisconsin 53233.

(7) Wunz, T. M.; Dorr, R. T.; Alberts, D. S.; Tunget, C. L.; Einspahr, J.; Milton, S.; Remers, W. A. *J. Med. Chem.* **1987**, *30*, 1313.

(8) Characterization data. Compound **1** (isolated as an HCl salt): mp 210 °C dec; UV (λ_{max}[pH 7]) 368 nm (ϵ 7300 M^{-1} cm^{-1}); ^1H NMR (D_2O) δ 1.95 (m, 6, CH_2), 3.1 (m, 12, CH_2), 5.25 (s, 2, Ar-CH_2-N), 7.6 (m, 4 ArH), 8.1 (d, 2, ArH), 8.24 (d, 2, ArH), 8.7 (s, 1, ArH); ^{13}C NMR (D_2O) δ 21.87, 22.82, 23.58 (imp), 37.56, 44.19, 45.56 (imp), 45.88, 50.80, 51.02, 121.79, 123.58, 126.65, 128.92, 130.55, 131.47, 131.70, 132.01; FAB mass spectrum, *m/e* 379 $(M + 1)^+$; high-resolution mass spectrum calcd for $C_{24}H_{35}N_4$ 379.286, and measured 379.285. Compound **2** (obtained as an HCl salt): mp 115 °C dec; UV (λ_{max}[pH 7]) 374 nm (ϵ 7100 M^{-1} cm^{-1}); ^1H NMR (D_2O) δ 2.15 (m, 12, CH_2), 3.1 (m, 24, CH_2), 5.38 (s, 4, Ar-CH_2-N), 7.73 (m, 4, ArH), 8.41 (m, 4, ArH); ^{13}C NMR (D_2O) δ 23.53, 24.52, 39.35, 47.58, 47.68, 52.91, 52.96, 126.75, 126.89, 130.60, 132.15; FAB mass spectrum, *m/e* 579 $(M + 1)^+$, 391 $(M - trpn)^+$; high-resolution mass spectrum calcd for $C_{34}H_{59}N_8$ 579.486, and measured 579.483.

(9) (a) Chandross, E. A.; Thomas, H. T. *Chem. Phys. Lett.* **1971**, *9*, 393. (b) Brimage, D. R. G.; Davidson, R. S. *J. Chem. Soc., Chem. Commun.* **1971**, 1385. (c) de Silva, A. P.; de Silva, S. A. *J. Chem. Soc., Chem. Commun.* **1986**, 1709. (d) Results using *N*-(9-anthrylmethyl)piprazine (ref 3).

(10) The known pK_a's for the conjugate acids of tris(3-aminopropyl)amine (*Inorg. Chem.* **1968**, *7*, 865) along with the perturbation of bonding a primary amine to a benzylic position allow us to estimate that 70% of **1** exists as the trication at pH 6.

(11) At pH 6 phosphate dianion is a minor ionic form (6%) in solution; however, we depict binding of the dianion based on the well-documented dependence of binding to polyamines on electrostatic forces. For a discussion of this subject see: Kimura, K. Macrocyclic Polyamines as Biological Cation and Anion Complexones—An Application to Calculi Dissolution. In *Biomimetic and Bioorganic Chemistry*; Boschke, F. L., Ed.; Springer-Verlag: New York, NY, 1985.

(12) The observation of CHEF upon acetate or sulfate binding does not refute the mechanism put forward in Figure 2. Intracomplex protonation (or strong hydrogen bonding) of **5** by the HPO_4^{2-} ion as shown in Figure 2 is *indistinguishable* from the binding of **3** to the PO_4^{3-} ion with subsequent enhanced benzylic amine protonation from the solvent. Such enhanced protonation could result from either (a) enhanced basicity of amine groups upon ion pairing of neighboring ammonium ions to the anion or (b) enhanced amine protonation resulting from intracomplex hydrogen bonding of the ammonium ion to a nearby guest oxygen (e.g., **6**). If a significant concentration of PO_4^{3-} was present at pH 6, one would anticipate a CHEF effect upon its binding to **3**. Fluorescence enhancement upon the binding of acetate, sulfate, or dimethyl phosphate can likewise result from effects (a) and (b), which differ from that shown in **6** prinicpally in the basicity of the bound anion.

(13) The pH of solutions was adjusted to 6; all solutions contained 0.1 M MES buffer.

(14) Pesce, A. J.; Rosen, C. G.; Pasby, T. L. *Fluorescence Spectroscopy*; Marcel Dekker, Inc.: New York, NY, 1971; Chapter 7.

(15) Binding constants were calculated with use of the total phosphate concentrations rather than the amount of any one ionic form and thus may best be seen as lower limits. Because of the relatively weak binding of acetate, dimethyl phosphate (both [−]1), and phosphate (94% [−]1, 6% [−]2 at pH 6) to **1**, binding saturation was not achieved in these cases. The percent CHEF reported is that for the most concentrated solution we were able to prepare, and the log K_{eq}'s are those obtained by iterative best fits of the data.

(16) Besides the ammonium ion in MES (ref 13), the only other cation present in each experiment was the tetrabutylammonium ion (except for the ATP titration, which contained a small amount of sodium ion). Up to a concentration of 0.2 M, tetrabutylammonium chloride does *not* change the fluorescence spectrum of **1** at pH 6.

0.82) at pH 6 along with the corresponding percent fluorescence intensity increase (>145%) could then be obtained.[17] Similarly, values were determined at pH 6 for the binding of **1** to ATP (log K_{eq} = 4.2, 79%), citrate (log K_{eq} = 2.3, 97%), sulfate (log K_{eq} = 1.6, 114%), acetate (log $K_{eq} \leq 0.6$, >98%), and dimethyl phosphate (log $K_{eq} \leq 0.5$, >66%). As an indication that even larger fluorescence enhancements are likely with structurally modified conjugate probes, we have observed a 6-fold CHEF effect for the binding of citrate to anthrylbis(polyamine) **2** (Figure 3); however, calculations indicate a binding event of more complex stoichiometry that is under study.

The present work demonstrates that intracomplex protonation of a quenching nitrogen leads to CHEF effects in aqueous solution in the same way that metal ion chelation does. We believe our results suggest a general and heretofore undescribed method for the chromogenic "signalling" of anion binding. Since the origin of the effect can be rationalized at the molecular level, a structural basis exists for the design of conjugate probes for ionic and hydrogen-bonding guests. Given the almost limitless synthetic approaches to nitrogen-containing hosts,[18] the fabrication of useful analytic tools seems likely to result.

Acknowledgment. We gratefully acknowledge support for this work from The National Science Foundation, and from The Ohio State University and Amoco in the form of graduate fellowships to one of us (M.E.H.). Shared resources, including the use of a Perkin-Elmer LS-5 Fluorimeter, were made available by Prof. M. Platz of this department. FT-NMR spectra were obtained with equipment funded in part by NIH Grant 1 S10 RR01458-01A1. A.W.C. thanks the A. P. Sloan Foundation for support in the form of a Fellowship and Eli Lilly and Co. and Merck for support in the form of Granteeships.

(17) Binding constants were determined by using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2, 1LA, United Kingdom.

(18) For an excellent overview of the great variation achievable in the design of polyammonium receptors, see: Schmidtchen, F. P. *Nachr. Chem., Tech. Lab.* **1988**, *8*, 10.

Chiral Organosilicon Compounds in Synthesis. Highly Enantioselective Synthesis of Arylcarbinols[1]

T. H. Chan* and P. Pellon

Department of Chemistry, McGill University Montreal, Quebec, Canada, H3A 2K6

Received June 21, 1989

Because of the usefulness of organosilicon compounds in organic synthesis, it is not surprising that considerable attention has recently been focused on the use of chiral organosilicon compounds for enantioselective synthesis.[1-11] One approach is to utilize organosilanes where the silicon atom is the chiral center, usually the 1-naphthylphenylmethylsilyl group.[2-7] The alternative approach is to use organosilicon compounds with the chirality located at a site attached to, but removed, from silicon. A number of groups,[8-11] including our own,[1] have taken up this approach because of the greater structural variety that can be incorporated into the chiral moiety. Chiral auxiliaries derived from optically active natural products[1,8,9,11] or by resolution[10] have been used. However, it is fair to say that the stereoselectivity obtained from either approach has been modest so far.

We report here our recent results, which show that highly enantioselective synthesis of arylcarbinols can be achieved by alkylation of chiral organosilicon compounds. It is our expectation that the approach may have general applicability.

The chiral organosilicon compound **1** was prepared from dimethyl(chloromethyl)benzylsilane (**2**)[12] and (*S*)-(+)-2-(methoxymethyl)pyrrolidine (**3**) (Scheme I). Treatment of **1** with *sec*-butyllithium in THF gave the carbanion **4**, which on quenching with methyl iodide gave the alkylated product **5a** in good yield. As is evident from ^{1}H NMR and subsequent transformations (vide

Scheme I

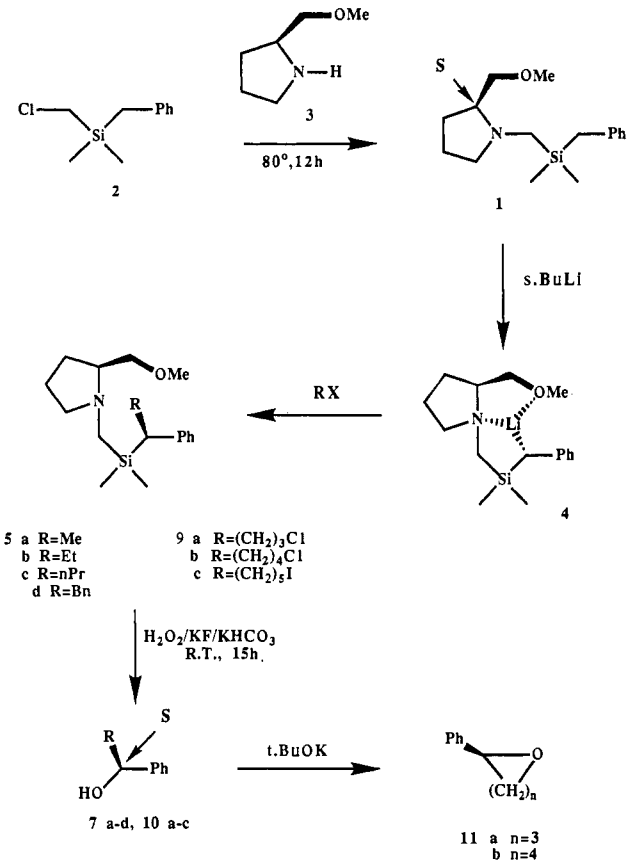

Table I. Alkylation of Carbanion **4** with Alkyl Halide RX According to Scheme I

RX	product	yield,[a] %	$[\alpha]^{20}_D$ (*c* 1, $CDCl_3$), deg	de (NMR),[b] %
MeI	**5a**	86	-98	>95
nPrI	**5c**	82	-73	>95
EtI	**5b**	78	-81	>95
$PhCH_2Cl$	**5d**	58	-4	>95
$I(CH_2)_5I$	**9c**	55	-43	>95
$Cl(CH_2)_3Br$	**9a**	61	-49	>95
$Cl(CH_2)_4Br$	**9b**	64	-59	>95

[a] Yield after flash chromatography. [b] ^{1}H NMR showed only one diastereomer.

(1) For previous publications on this subject from this laboratory, see: Chan, T. H.; Wang, D. *Tetrahedron Lett.* **1989**, *30*, 3041.

(2) Brook, A. G.; Duff, J. M.; Anderson, D. G. *J. Am. Chem. Soc.* **1970**, *92*, 7567.

(3) Torres, E.; Larson, G. L.; McGarvey, G. J. *Tetrahedron Lett.* **1988**, *29*, 1355.

(4) Bonini, B. F.; Mazzanti, G.; Zani, P.; Maccagnani, G. *J. Chem. Soc., Chem. Commun.* **1988**, 165.

(5) Hathaway, S. J.; Paquette, L. A. *J. Org. Chem.* **1983**, *48*, 3351.

(6) Fry, J. L.; McAdam, M. A. *Tetrahedron Lett.* **1984**, *25*, 5859.

(7) Stang, P. J.; Learned, A. E. *J. Org. Chem.* **1989**, *54*, 1779.

(8) Walkup, R. D.; Obeyesekere, N. U. *J. Org. Chem.* **1988**, *53*, 923.

(9) Cappi, L.; Mordini, A.; Taddei, M. *Tetrahedron Lett.* **1987**, *28*, 969.

(10) Jung, M. E.; Hogan, K. T. *Tetrahedron Lett.* **1988**, *29*, 6199.

(11) Tamao, K.; Kanatami, R.; Nada, M. K. *Tetrahedron Lett.* **1984**, *25*, 1913.

(12) Compound **2** was prepared from the reaction of benzylmagnesium chloride and dimethyl(chloromethyl)chlorosilane in 95% yield. It has the following physical characteristics: bp 120-124 °C (40 mmHg); ^{1}H NMR (in $CDCl_3$, 200 MHz) δ 7.20 (s, 5 H), 2.78 (s, 2 H), 2.27 (s, 2 H), 0.15 (s, 6 H).

0002-7863/89/1511-8737$01.50/0